Biochemical Engineering Journal 56 (2011) 142–149

Contents lists available at ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Preparation of carriers based on magnetic nanoparticles grafted polymer and immobilization for lipase

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ARTICLE INFO

Article history:
Received 18 March 2011
Received in revised form 17 May 2011
Accepted 28 May 2011
Available online 6 June 2011

Keywords:
Super paramagnetism
Electrostatic adsorption
Covalent binding
Immobilized CRL
Enzyme technology
Reusability

ABSTRACT

The super paramagnetic nanoparticles Fe_3O_4/(2-hydroxyethty methacrylate-co-dimethyl dially ammonium chloride) copolymer, i.e. Fe_3O_4/P(HEMA–DMDAAC), with positive charge and active epoxy groups simultaneously, were synthesized by a surface-initiated radical polymerization and activation reaction with epoxy chloropropane. The production particles were characterized by Fourier transform infrared spectroscopy (FT-IR), thermogravimetric analysis (TG), transmission electron microscopy (TEM), and vibrating sample magnetometry (VSM). It was verified that the magnetic microspheres held small diameters of 80–100 nm and displayed super paramagnetic property with saturation magnetization of 38.9 emu/g. The candida rugosa lipase (CRL), meanwhile, was immobilized onto the magnetic microspheres via electrostatic adsorption and covalent binding, the loading amount of lipase was 68.3 ± 0.5 mg CRL/g support and the activity recovery of the obtained immobilized lipase reached to 60.4% (±1.6%).

1. Introduction

Enzyme, as well known, is preferred to chemical catalyst because of its high effectiveness, high specificity and mild reaction conditions [1]. In recent years, enzyme has become a viable alternative for many applications [2]. Lipase (triacylglycerol acyl hydrolases) is a kind of ubiquitous enzymes with various biological activities, including enantioselective hydrolysis and esterification, chiral resolution, synthesis of enantioenriched monomers and macromolecules for polymerization reactions, and other enzymatic reactions [3–5]. Candida rugosa lipase (CRL), among the lipases from various sources, received much attention due to its high activity and broad specificity [6]. However, the industrial applications of biocatalysts have not yet reached a significant level due to the high costs of the enzymes and the inability in their separation, recycling, and reusing [7]. Thus, the technology of enzyme immobilization, which could achieve the reusability of enzyme and prove the stability of enzyme [8], was developed to overcome these inconveniences [9].

Until now, a variety of nano-sized particles have been used to immobilize enzyme [10]. However, it is difficult to recover small immobilized enzyme particles from reaction system using conventional carriers. In view of the above disadvantages, functional super paramagnetic iron oxides have attracted great interest in enzyme immobilization [11]. On one hand, the functional magnetic microspheres can be covalently binded with different groups (e.g. amino, hydroxyl, or thiol moieties) on the protein surface to form strong linkages, which can prove the stabilities of enzymes [12]. On the other hand, enzymes immobilized by magnetic microspheres could be separated easily and rapidly from the reaction system to enhance the reusability of enzyme [13], and they can also be stabilized in a fluidized-bed reactor by applying an external magnetic field [14,15]. In order to increase the loading amount of enzyme on magnetic particles and improve the stability of immobilized enzyme, different organic macromolecules has been used to functionalizing the surface of magnetic particles, such as glycidyl methacrylate (GMA), methyl methacrylate (MMA), 2-hydroxyethty methacrylate (HEMA), methacryloxyethyl trimethyl ammonium chloride (MATAC), etc. [16–18]. During the process of enzyme immobilization, different methods could be adopted to immobilize enzyme, such as covalent binding [11], entrapment [19] or adsorption [20], etc. However, it has been proved that immobilization with two methods would greatly increase the enzyme loading amount and improve the stability of the enzyme [21]. Thus, the carrier, which has positive charge and active groups (such as epoxy group, amino group, etc.) simultaneously, would be a kind of efficient supports.

In this paper, super paramagnetic Fe_3O_4 nanoparticles modified by vinyltriethoxysilicane (VTES) were prepared firstly.

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1369-703X/$ – see front matter.
doi:10.1016/j.bej.2011.05.013

Then, 2-hydroxyethty methacrylate (HEMA) and dimethyl dially ammonium chloride (DMDAAC) were grafted onto modified nanoparticles by surface-initiated radical polymerization. At last, the magnetic nanoparticles were activated by epoxy chloropropane. HEMA is well known for its non-toxicity, biocompatibility and widespread biomedical applications [22], while DMDAAC can provide the carriers positive charge. Thus, candida rugosa lipase (CRL) was immobilized on the resulting magnetic microspheres by electrostatic adsorption and covalent binding. The structure and properties of the magnetic carriers were characterized by Fourier transform infrared spectroscopy (FT-IR), thermogravimetric analysis (TG), transmission electron microscopy (TEM), and vibrating sample magnetometry (VSM). Additionally, the optimal conditions of immobilizing lipase were determined and the properties of the immobilized lipase (such as activity recovery, thermal stability reusability and storage ability) were investigated.

2. Chemicals and methods

2.1. Chemicals

2-Hydroxyethty methacrylate (HEMA) and dimethyl dially ammonium chloride (DMDAAC) were obtained from Ciba Specialty Chemicals (China) Ltd., Guangzhou; vinyl triethoxy silane (VTES) was chemical grade and purchased from Wuhan New Materials Co.; azobisisobutyronitrile (AIBN) was chemical grade and purchased from Forth Shanghai Reagent Factory (China); candida rugosa lipase (CRL), Type VII, 1180 units/mg (solid) and bovine serum albumin (BSA) were analytic grade and purchased from Sigma Chemical Co.; other chemicals and solvents were all of analytical grade and obtained from Tianjing Chemical Reagent Company (China). All the reagents of chemical grade were used with further purification.

2.2. Preparation of Fe_3O_4 nanoparticles

Fe_3O_4 nanoparticles were prepared by a chemical co-precipitation method described previously [23] with some modifications. 35 ml of 1 M $FeCl_2$ and 1.5 M $FeCl_3$ solution were added into a three-necked flask to make the molar ratio of Fe^{2+} and Fe^{3+} maintaining 1:1.5, and stirred together under nitrogen. When the solution was heated to 60 °C, ammonia (25%, w/w) was added to regulate the pH value of reaction system to 10–11, and solution became dark after base addition. Then the solution was heated at 80 °C for 1 h with vigorous stirring. After solution cooled, the precipitates were isolated from reaction system and washed several times with distilled water until the solution became neutral. Finally, the resulting magnetic nanoparticles were obtained after dried at room temperature under vacuum.

2.3. Modification of Fe_3O_4 nanoparticles with VTES

Modification of Fe_3O_4 nanoparticles with VTES is achieved by the reaction between the hydroxyl groups of the hydrolytic VTES and the hydroxyl groups on the surface of magnetite. 1.0 g of Fe_3O_4 nanoparticles were dispersed in 60 ml of ethanol and vibrated with ultrasonic for 1 h, then certain $NH_3{\cdot}H_2O$ ($NH_3{\cdot}H_2O$:VTES = 2:1, v/v) was added and ultrasonic vibrated to homogenize for 10 min. With continuous mechanical stirring, 6.0 ml of VTES was added into the reaction mixture. The reaction was heated at 50 °C for 8 h under continuous stirring and nitrogen atmosphere. At last, the products were separated by permanent magnet and then washed thoroughly with ethanol and distilled water until neutral. Finally, the VTES-modified magnetic nanoparticles were obtained after dried at room temperature under vacuum.

2.4. Preparation of magnetic Fe_3O_4/P(HEMA–DMDAAC) nanoparticles

The magnetic polymeric nanoparticles were synthesized according to the following steps: 0.5 g of VTES-modified Fe_3O_4 nanoparticles, certain KPS and $NaHSO_4$ with molar ratio of 1:1, 44 ml of ethanol and 10 ml of distilled water were put in a three-necked flask and vibrated with ultrasonic for 30 min under nitrogen to be dispersed uniformly. After these mixtures were placed at 4 °C overnight, the flask was moved into a water bath at 70 °C and mechanically stirred under a nitrogen atmosphere. Then a mixture of HEMA (4.8 ml, 0.04 mol), DMDAAC (10% of the total weight of monomers), ethanol (7 ml) and deionized water (7 ml) was added dropwise into the flask in 1 h. After this, the polymerization was taken out at 70 °C for 6 h. At last, the products were isolated from solution by magnetic decantation and washed repeatedly with distilled water and ethanol. Then the nanoparticles were extracted with ethanol in soxhlet extraction for 48 h and dried at room temperature in vacuum drying oven for 24 h.

2.5. Activation of magnetic Fe_3O_4/P(HEMA–DMDAAC) nanoparticles

The hydroxyl group could not combine with lipase efficiently, so the magnetic polymer carriers should be activated by epoxy chloropropane. 4 g of magnetic Fe_3O_4/P(HEMA–DMDAAC) nanoparticles were swelled in dimethyl sulfoxide overnight. Then 16 ml of epoxy chloropropane were added into the mixture and the reactants were heated to 40 °C, and 40 ml of NaOH solution (3 mol/l) were added drop-wise into the reaction system. After 4 h, the reaction was finished. The activated products were filtered and washed several times with distilled water until the solution became neutral. Finally, the resulting magnetic nanoparticles were obtained after dried at room temperature under vacuum.

Scheme 1 shows the preparation of magnetic epoxy supports including the synthesis of magnetic Fe_3O_4/P(HEMA–DMDAAC) nanoparticles and the activation of magnetic supports.

2.6. Characterization of magnetic polymer supports

FT-IR spectra from the KBr pellet with OA-Fe_3O_4 and the magnetic polymer microspheres, respectively, were recorded by a Fourier Transform infrared spectrophotometer (Nicole NEXUS 670, USA).

Thermogravimetric analysis was observed by a TG-DSC apparatus (NETZSCH STA 449C) by heating the samples from room temperature to 900 °C under Ar atmosphere at a heating rate of 20.0 K/min.

The morphologies of magnetic polymer microspheres were observed by a transmission electron microscopy (TEM, FEI Tecnai G20).

The magnetization curves of the magnetic polymer microspheres were measured with a vibrating sample magnetometer (LAKESHORE-7304, USA) at room temperature.

2.7. Lipase immobilization

The obtained Fe_3O_4/P(HEMA–DMDAAC) supports were used for immobilizing lipase. On one hand, this composite support has good adsorption effect for lipase because of the positive electrical charges on the surface of supports. On the other, the active epoxy groups in the activated magnetic polymer supports can react with the amino group of lipase in gentle conditions [24,25]. Therefore, the lipase immobilization was carried out by reaction of lipase solution with magnetic polymer supports directly. Firstly, 1 g of magnetic nanoparticles supports had been dipped in 50 ml of phos-